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INFECTION OF BIRDS WITH PROTEOSOMA BY THE BITES OF MOSQUITOES.

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ON SPECIAL DUTY.

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INFECTION OF BIRDS WITH PROTEOSOMA BY THE BITES OF MOSQUITOES.

By Major RONALD ROSS, I. M. S.,
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1. *Preliminary.*—In a report, dated the 21st May 1898, on the cultivation of Proteosoma, Labbé, in grey mosquitoes[1] I showed that two of the gymnosporidia, namely, proteosoma of birds and one of the crescent-bearing species of man, were capable of development respectively in "grey" and in "dappled-winged" mosquitoes. It was demonstrated that the derived stage of the parasites in the mosquitoes begins life (so far as ascertained) as a small, oval, pigmented cell lying in or close to the outer muscular coat of the stomach of insects fed on birds or men infected with the gymnosporidia, and that the cells increase day by day in size, gradually losing their pigment and at the same time becoming protruded from the viscus into the

[1 Published in *The Indian Medical Gazette* of 1898, p. 448, *et seq.*—Ed., *I. M. G.*]

cœlom or blood cavity of the insect, until at the end of about a week (in the case of proteosoma) they reach a diameter of about 60μ or more, and are evidently ready for some form of sporulation. I considered these bodies to be *coccidia*,[1] and held that a knowledge of their further life-history would contain the solution of the problem of the mode of propagation of malaria. The report referred to, however, dealt only with the subject-matter under its title, that is, with the facts showing the cultivation of these gymnosporidia in mosquitoes to be possible, and made no attempt to trace their development beyond a certain point.

The present report, dealing briefly with some later researches on the same subject, may I think be called for as a preliminary to a more detailed exposition which I hope to submit at a future date.

2. *Reproduction-forms of the Proteosoma-Coccidia.*— To take up the subject again, then, at the point at which the former report abandoned it—the examination of a very large number of grey mosquitoes fed on sparrows infected with proteosoma showed clearly that the proteosoma-coccidia after reaching maturity (seventh day) form two different kinds of reproductive elements, namely (*a*) a large number of delicate thread-like bodies, or (*b*) a smaller number of large black spores.

The thread-like bodies are from 12μ to 16μ in length, about 1μ in breadth, and are flattened in the third dimension. From the middle, which contains vacuoles and chromatin granules, they taper to each extremity; and when viewed

[1] This is conjectural and provisional. The true zoological significance of the mosquito stage of the parasites is a difficult subject, and will be discussed in the final report.

under the best conditions they are not unlike small trypanosomes, though I have never succeeded in detecting any certain indications of movement in them. They are closely packed in thousands within the capsule of the mother coccidium. Plate I, figures 18 and 19 of my former report represent two "striated" coccidia which in reality were coccidia containing these elements. On rupturing such a body by pressure on the cover-glass the thread-like elements can be seen pouring out in vast numbers and floating away in the surrounding fluid.

The *large, black spores*, when mature, are from 16μ to 20μ or more in length and 2μ or 3μ in thickness. They are cylindrical, closed at the ends, and are straight, curved, sigmoid or variously twisted. Their colour is a dark brown, they have a black contour, and they are evidently capable of resistance to outer forces. Plate I, figure 20 of the former report depicts several of them contained within a coccidium. It is difficult to estimate the number of them produced by each parasite, as many appear to be ejected before being fully matured.

I have never observed both the thread-like elements and the black spores in the same coccidium. It appears likely that the *hyaline cells* described in the previous report produce the former, and the *vacuolated cells* the latter.

3. *Escape of the Reproduction Forms.*—On the eighth or ninth day, that is shortly after the coccidia have matured and produced their reproductive elements, they burst *in situ* in the living insect and pour those elements into the general cavity of the body, which contains the so-called *blood* or circulating juices. The empty capsule of the coccidium remains behind still attached to the wall of the stomach; but the reproductive

elements are swept away by the blood-current and distributed throughout the tissues of the mosquito.

If, for example, we kill a mosquito ten days after it was fed on a sparrow with proteosoma, we shall almost certainly find large numbers of the thread-like bodies in the juices of the head and thorax, and similarly, some of the black spores in almost all the muscular and connective tissues of the body.

4. *Further migration of the Thread-like Elements.*—It has long been found difficult to explain the irritation of mosquito-bites. While many have supposed it due to the injection of some acrid fluid by the insect, no one, so far as I know, has yet succeeded in demonstrating the existence of any such poison. It has remained to the parasites of malaria to give, so to speak, this demonstration. While examining the thread-like elements in the blood of mosquitoes I observed that they were to be frequently found collected within the cells of some gland in the thorax, the nature of which was unknown to me at the time. This gland eventually proved to be the *salivary* or poison gland of the mosquito.

Closely similar to the well known salivary gland of other insects, this organ lies in the neck or the anterior part of the thorax of the mosquito and consists of a number of separate lobes. Each lobe is made up of numerous large cells clustered round a central duct and contained within a limiting membrane. The ducts of the several lobes ultimately unite and form a single main effluent. This runs up the under-surface of the head in the middle line; enters the base of one of the stylets or lancets of the proboscis, namely, the central unpaired one,

called the tongue or epipharynx ; traverses the whole length of it and opens at its extremity in such a manner that the secretion of the gland must be poured into the very bottom of the wound made by the piercing apparatus of the proboscis.

It is in the cells of this gland that the thread-like reproductive elements of the proteosoma-coccidia have the power of accumulating. How they manage to enter the cells from the blood I do not know; but it is certain that they can be found in them in large numbers, either floating separately in the grape-like cavity of the cells, or crowded together within them in hundreds. It is impossible to mistake their identity.

Generally only one or two of the lobes of the gland contain these elements, the other lobes being free from them. Often, as I estimate, one lobe must contain many thousands.

5. *Interpretation of these facts.*—It is, I think, impossible to err in the interpretation of these observations. The secretion of the gland is obviously meant to be injected into the wound made by the tongue and the other stylets. Its function is, I apprehend, not to cause irritation —which of course would be worse than useless to the insect, but to check the spasmodic contraction of the torn capillaries, which would otherwise quickly stay the flow of blood into the wound. The thread-like elements seize the opportunity, so to speak, of the existence of this gland for the purpose of returning to a warm-blooded host. Penetrating into the secreting cells of the gland they remain there, waiting the opportunity when they shall be carried, together with the secretion, directly into the blood-vessels of the victim of their mosquito

host. There, if that victim happen to be a bird of an amenable species, they doubtless originate an infection of proteosoma, thus completing the cycle of the parasite.

6. *Infection of Birds by the Bites of Mosquitoes.*—All that was now required to obtain complete proof of this theory was actually to infect healthy birds in the manner suggested. This was accomplished very easily. At the end of last June, four sparrows and one weaver bird, whose blood on several examinations had been found to be entirely free from proteosoma, were subjected nightly to the bites of numerous grey mosquitoes fed more than a week previously on a sparrow containing proteosoma. On the 9th July these five birds were examined and were now found to have become infected with swarms of the parasites. All of them died very soon, and their liver and spleen were seen to be profusely charged with the characteristic black pigment of malaria.

The experiment was next repeated over and over again on a number of sparrows and other birds. A large percentage of these became infected after a definite period of incubation.

Thus out of 28 originally healthy sparrows subjected to the bites of grey mosquitoes previously fed on diseased sparrows, 22 or 79 per cent., became infected, all with a very large number of parasites, in from five to eight days. This excludes a number of birds which died before the end of the incubation period from diarrhœa and other disease to which sparrows in captivity in Calcutta are subject. Out of the six birds which failed to become infected after these experiments, one was subjected to a second trial, which gave a successful result.

Again, out of two crows and four weaver birds some of which contained halteridium though

none contained proteosoma, one of the crows and all the weaver birds showed a copious proteosoma infection within nine or ten days of being bitten by grey mosquitoes fed previously on sparrows with the latter parasite.

Lastly, out of five sparrows which originally contained a very few proteosoma, four showed a new and much more copious infection a week after being subjected to a similar experiment.

I failed, however, to transmit the proteosoma of sparrows to *mainas* and some other birds.

In all the birds in which the parasites appeared after the experiment, the invasion presented such constant and unmistakable characters that no possible room for doubt was left as to the infection being due to the mosquitoes. The blood of the birds experimented with was examined both before the experiment and on several occasions afterwards. The course of events was always as follows. The blood would remain entirely free from proteosoma (except in the case of the five sparrows which originally contained a few of them) until the fifth, sixth, seventh or eighth day after the experiment, when one or two parasites only would be found in an entire specimen. Next day it would invariably be seen that the number of parasites had largely increased; and this increase would continue until in a few days, in almost every case, the parasites become so exceedingly numerous that from ten to sixty and even more could be counted in almost every field of the oil-immersion lens, while I often observed as many as seven distinct parasites in a single corpuscle. The only exceptions were the case of the crow and that of the five originally infected sparrows, in which the number of parasites did not exceed about

one in each field, even this being a high figure for proteosoma.

Most of the birds now died, and showed not only the characteristic pigmentation of the liver and spleen, but also a distinct inky colour in the blood itself. In the few which recovered, however, the number of parasites rapidly declined.

It is necessary to compare with these results those obtained from the examination of wild sparrows caught in Calcutta. Out of 111 of these I found proteosoma in 15 only, or in 13·5 per cent., and in most of these the parasites were very few in number, exceeding in only two of them an average of one to each field. Hence, in the infections artificially induced by me both the percentage of birds affected and the number of parasites found were so largely in excess of the normal rate that, apart from other reasons, it is impossible to question the fact of proteosoma being communicable by mosquitoes.[1]

7. *Communicability of Human Malaria.*—I have not been able to study the reproductive elements of the mosquito stage of the human gymnosporidia; but there is no reason to suppose that they differ much from those of the

[1] Control experiments were not absolutely necessary in these experiments. The gradual invasion of the blood by the parasites after a definite incubation period, the absence at first of forms containing discrete pigment, the enormous number of parasites finally observed, and the large percentage of birds in which these results were attained, sufficed to establish this law of communication. Nevertheless, a large number of healthy sparrows were kept as controls, being preserved from accidental infection from the bites of free mosquitoes in the laboratory by being placed every night in their cages within a mosquito netting. Only one of these birds was found to have become infected on a second examination; and, in this case, the parasites were so few that I think I must have overlooked them on the first observation.

proteosoma coccidia, or that the modes of infection of human malaria are unlike those of avian malaria.

8. *Function of Black Spores.*—This also remains undetermined. They have failed to infect several sparrows to which numerous mosquitoes containing them were administered by the mouth; they have also failed (?) to infect mosquito larvæ when given in a similar manner, and have undergone no change when kept in the damp chamber for seven weeks.

9. *Other Questions.*—Further details of these observations, together with drawings and photographs, are reserved for a future report, in which also I hope to deal, at least in part, with the conjugation of the smaller elements of proteosoma, the communicability of the parasites from one species of bird to another, immunity, and other questions now open to study.